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AGRIC. BIOL. CHEM., vol. 49, no. 2, February 1985, pages 541-543, The Agricultural Chemical Society of Japan; T. IKEDA et al.: "Glucose oxidase-immobilized benzoquinone-carbon paste electrode as a glucose sensor"

AGRIC. BIOL. CHEM., vol. 48, no. 8, 1984, pages 1969-1976; T. IKEDA et al.: "Electrocatalysis with a glucose-oxidase-immobilized graphite electrode"

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EP 0 177 743 B1

Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an enzyme electrode comprising a carrier, an enzyme immobilized on a part of the outer surface of the carrier, a coating film consisting of a thin film permeable for a substrate for the enzyme and coating the portion where the enzyme is immobilized, and an internal electrode capable of applying voltage to the portion.

More particularly, it relates to electrodes for amperometrically measuring concentrations in an electrolyte of those substances which can be the substrate for oxidases, such as glucose, galactose and the like, or of those substances which can be the substrate for dehydrogenases, such as lactic acid, alcohol, glycerol and the like.

2. Description of the Prior Art

In recent years, increasing attention has been paid to oxidoreductase immobilized electrodes (hereinafter, referred to as enzyme electrodes) (reference is made to T. Ikeda, S. Ando, and M. Senda, Bull. Chem. Soc. Jpn., 54, 2189 (1981)) These electrodes behave as a substitute for a chemical electron transporter in enzyme reaction, and it has been suggested that they could be utilized in such novel applications as enzyme electrodes, detectors in flowing system, biochemical fuel cells and enzyme reactors (reference is made to S. Ando, T. Ikeda, T. Kakutani, and M. Senda, Rev. Polarogr. (Kyoto), 26, 19(1980): abstracts of papers presented at the Annual Meeting on Polarography, October 1980, Fukuoka).

Such enzyme electrodes are of the form directly utilizing current (electric current) and voltage related to enzyme reaction, being different from those electrodes of the form indirectly estimating the amount of substrate contributing to enzyme reaction by measuring the amount of a substance produced in enzyme reaction, for example, the amount of hydrogen peroxide generated, with the so-called oxygen electrode or platinum electrode whose sensitive surface is coated with an enzyme-immobilized film.

In this regard, the inventors of the present invention have previously found that glucose oxidase-immobilized electrodes using graphite as carrier can function as a bioelectrocatalysis electrode for electro-oxidation of glucose in the presence of an electron transfer mediator such as p-benzoquinone in solution (reference is made to T. Ikeda, I. Katasho, M. Kamei, and M. Senda, Agric. Biol. Chem., 48, (8) (1984)).

In such oxidase electrodes, however, there were problems on practical use. For example, (a) it was requested to add to the solution to be measured a substance working as the electron transfer mediator participating in enzyme reaction, at every time of measurement, (b) since the amount of such substance capable of contributing to the reaction was regulated by its concentration in the solution and moved by concentration polarization during electrolysis, the substance could not be supplied to the reaction system in a high and constant concentration and accordingly the response speed and the sensitivity were insufficient, and (c) the influence on the substance of these substances coexisting in the solution to be measured, pH of the solution, oxygen contained in the solution, etc., as well as that of light, could not be disregarded.

On the other hand, it has been also suggested to form an oxidation-reduction system by utilizing immobilized dehydrogenase in combination with nicotinamide adenine dinucleotide (NAD) as electron transfer mediator and use the system for electrode reaction. Moreover, in order to carry out the measurement without adding the NAD to the solution to be measured at every time of measurement as described above, it has been tried to immobilize NAD together with enzyme. As representatives of such trials, ① electrodes prepared by trapping a NAD-high molecular compound obtained by chemically binding NAD with a high molecular compound, such as agarose or dextran, on the inside surface of a suitable substrate-permeable film together with enzyme and fixing the film to a platinum or graphite electrode, ② electrodes prepared by chemically binding NAD directly with a substrate-permeable film, trapping enzyme on the inside surface of the film simultaneously and fixing the film to a platinum or graphite electrode, ③ electrodes prepared by applying chemical treatment to a substrate-permeable film to make it hardly permeable one for NAD, trapping enzyme on the inside surface of the film and fixing the film to a platinum or graphite electrode, and the like have been suggested.

However, the above-described enzyme electrodes ① and ② involved the problem that the enzyme reactivity of NAD itself was largely decreased by conversion of NAD into a high molecular compound, and the electrodes ③ involved the problem that leakage of NAD could not be prevented perfectly and the enzyme reactivity lowered as time passed.

US 4,224,125 discloses an enzyme electrode comprising an oxidoreductase and, where necessary, a coenzyme, a redox polymer acting as an electron mediator in an enzymatic reaction and an electron collector, wherein the oxidoreductase and the redox polymer are both maintained in the immobilized state on or in the neighborhood of the electron collector. The redox polymer and the coenzyme are covalently bonded to a carrier both are in an immobilized state. Hence, diffusion of the mediator is prevented by the covalent bond between the coenzyme and the redox compound to the carrier.

FR 2 152 219 discloses an enzyme electrode comprising an electrochemical sensor, a layer pertaining an enzyme and an acceptor being in contact with the electrochemical sensor and the semi-permeable membrane covering the layer. The acceptor is contained in the reservoir in a solubilized state; therefore, the acceptor in the solution may easily run away through the coating film into the test solution and supplies too much of the acceptor for the enzyme to the enzyme-immobilized surface.

EP 0 136 362 discloses a bio-sensor comprizing a measuring electrode in an adjacent electrode embedded in an insulating substrate. The exposed surface of the electrode system is coated with a porous substance carrying an oxidation reductase and, if needed, an electron acceptor in a dry state. Said porous substrate is a hydrophylic porous membrane, preferably are nylon non-woven fabric. This porous membrane being impregnated with a mediator and the enzyme does not act as a suitable storage layer for supplying the mediator over a long period. Furthermore, mediator and enzyme are not separated relating to space.

The present invention is one which is made in view of various problems as mentioned above, and one of its purposes is to provide enzyme electrodes capable of maintaining a high reactivity of immobilized enzyme for a long period of time without adding any electron transfer mediator to the solution to be measured.

SUMMARY OF THE INVENTION

Thus, the present invention provides an enzyme electrode comprising a carrier, an enzyme immobilized on a part of the outer surface of the carrier, a coating film consisting of a thin film permeable for a substrate for the enzyme and coating the portion where the enzyme is immobilized, and an internal electrode capable of applying voltage to the portion, said carrier being in the form of a porous material, paste or gel being mixed with a substance acting as electron transfer mediator to provide a storage layer for the mediator whereby the concentration of the mediator at said portion is able to keep substantially constant for a long period.

Such enzyme electrodes of the present invention are useful for measurement of concentrations of the substrate in vital samples such as blood serum, blood plasma, urine, etc. or in enzyme reactors, and also valuable as electrodes for enzyme reactors or fuel cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1,Fig. 6,Fig. 13 and Fig.16 are each a diagram showing the construction of an embodiment of enzyme electrodes of the present invention.

Fig. 2A and Fig. 2B are each a graph showing the cyclic voltammogram pertaining to the enzyme electrode of Example 1, A being for the case wherein glucose is not added and B being for the case wherein 41 mM glucose is added. In these Figures, the broken lines c and d are for electrodes not containing p-benzoquionone in paste (electrodes of Referential Examples) and the full lines a and b are for electrodes of the present invention.

Fig. 3A and Fig. 3B are each a graph showing the relation between glucose concentration and anode current Is at an applied voltage of 0.5 V, with regard to the electrode of Example 1. Fig. 4 is a graph showing the relation between glucose concentration and anode current Is at the same applied voltage of 0.5 V, the plot o being for a deaerated solution and the plot ● being for an air-saturated solution.

Fig. 5 is a graph showing the cyclic voltammogram of the glucose oxidase electrode of Example 2. Fig. 7 is a graph showing the cyclic voltammogram pertaining to the alcohol dehydrogenase electrode of Example 4, the full line being for an electrode containing NAD in paste and the broken line being for an electrode containing NADH.

Fig. 8 is a graph showing the relation between the steady state current Is and the ethanol concentration in the solution measured in Example 4. Fig. 9 is a graph showing the cyclic voltammogram of the glucose dehydrogenase electrode of Example 5, and Fig. 10 is a graph showing the responsibility between glucose concentration and anode current.

Fig. 11 is a graph showing the cyclic voltammogram pertaining to the enzyme electrode of Example 6,

and Fig. 12 is a graph showing the responsibility to the substrate.

Fig. 14 is a graph showing the cyclic coltammogram pertaining to the enzyme electrode of Example 7, and Fig. 15 is a graph showing the responsiblity to the substrate.

Fig. 17 is a graph showing the cyclic voltammogram pertaining to the enzyme electrode of Example 9, and Fig. 18 is a graph showing the responsiblity to the substrate.

Fig. 19 is a diagram showing the construction of the enzyme electrode of Example 10. Fig. 20 is a graph showing the cyclic voltammogram pertaining to the enzyme electrode of Example 10, and Fig. 21 is a graph showing the responsibility to the substrate.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The enzyme electrodes of the present invention are usually used by setting an electrolysis system wherein the enzyme electrodes are used to act as anode, and keeping an enzyme-immobilized surface (a substrate-sensitive surface) kept in contact with, or dipped in, the solution to be measured. That is, the enzyme electrodes of the present invention can be used as an amperometric electrode, especially as a sensor. In these cases, there is no special limitation on the cathode (the counter-electrode) , and various electrodes such as platinum electrode, silver/silver chloride electrode, mercury/mercurous chloride electrode, etc. can be used as the cathode. By the way, the measurement as sensor is usually effected according to the controlled-voltage electrolysis.

The electrodes of the present invention are usually in the form that a carrier is buried at a terminal portion of a cylindrical insulator as the supporting material for sensor, the substrate-sensitive portion is formed on the exposed surface of the carrier, and an internal electrode and lead wires are suitably provided. However, they may be in any other form (for example, the form that they are incorporated directly in a flowing passage) in accordance with their use, as far as the substrate-sensitive portion constitutes the electrode. As the case may be, the counter-electrode can be incorporated in the same supporting material for sensor.

The most characteristic feature of the present invention resides in the point that the carrier for immobilizing enzyme is mixed with a substance which can be the electron transfer mediator for enzyme reaction resulting in the carrier being impregnated with the mediator. As a result of such impregnation, the electron transfer mediator is well supplied in a high concentration to the enzyme-immobilized surface. Since the electron transfer mediator consumed by enzyme reaction is reproduced by electrode reaction, any net consumption does not occur by reaction. Further, even if the electron transfer mediator leaked out through the coating film for enzyme surface, it is supplied from the carrier as a storage layer for the transfer mediator in high concentration. Therefore, the concentration of the electron transfer mediator at the enzyme-immobilized surface is kept substantially constant. Thus, the intended enzyme reaction can be performed along with the electrode reaction without adding the electron transfer mediator to the solution to be measured, without any lowering in the enzyme reaction activity.

As the carrier of the enzyme electrodes of the present invention, one having at least a surface capable of fixing enzyme physically or chemically and being in the form capable of being impregnated with an electron transfer mediator as described hereinafter is selected. To be concrete, one in the form of porous material, paste or gel is used. As the porous material, it is preferable to use a porous molding of a conductive or semi-conductive substance, such as sintered mateiral or sponge, which is processed into the desired form. As the paste or the gel, it is preferred because of the easiness on forming the internal electrode to use a paste or gel containing a conductive or semi-conductive powder. As examples of the conductive substance, graphite and nobel metals such as gold, platinum, etc. are mentioned. As the semi-conductive substance, inorganic compounds such as titanium oxide, tin oxide, etc. are mentioned. Further, the so-called conductive high molecular compounds (high molecular electroconductive materials) can be used as the conductive or semi-conductive substance, and as examples of them there can be mentioned polyacetylenes, polypyrrole, polythiophene, polyselenophene, poly-p-phenylene sulfide poly-p-phenylene selenide, poly-p-phenylene oxide, polyaniline, etc. which are provided with a suitable conductivity by doping.

As preferred examples of the above porous molding, there can be mentioned porous graphite.

As preferred examples of the above paste or gel, there can be mentioned a paste or gel obtained by mixing graphite powder or a nobel metal powder with a non-polar binder such as liquid paraffine, undecane, Teflon paste, silicon grease, white vaseline, etc. and, if necessary, a viscosity-increasing agent such as cellulose powder and/or a high molecular gelling agent. The amount of the above conductive or semi-conductive substance powder is suitably 60 - 70 % by weight of the whole, in view of form or shape maintenance. The particle diameter of the powder is suitable about 1 μm - 50 μm.

However, even a paste or gel not containing any conductive or semi-conductive substance powder may be used as the carrier of the electrodes of the present invention, as is illustrated by Example 10. Likewise, the porous material may also be constituted with an insulating substance (such as resin or ceramics). It is also possible to use an aggregate of fibers such as carbon fibers as the carrier.

Such carrier acts as a sort of storage layer for the electron transfer mediator, and its capacity is properly decided according to the term of use and the easiness of treatment of the enzyme electrode.

Impregantion of the carrier as described above with a substance which acts as the electron transfer mediator can be performed easily when the carrier is a paste or gel, by directly admixing the substance to the paste or gel as it is prepared. On the other hand, when the carrier is a porous material, the impregnation can be performed easily by dipping the porous material in a liquid dispersion of the substance or preferably in a sol for the above-mentioned paste or gel, or by applying, the liquid dispersion or the sol to the porous mateiral. That is, the porous material is impregnated with the substance which acts as the electron transfer mediator, in the form of the above mentioned paste or gel. When an aggregate of fibers is used as the carrier, the impregnation in the form of paste or gel may be performed according to the same manner as that employed for the porous material.

As the electron transfer mediator with which the carrier as described above is impregnated, various compounds which participate in the reaction as an electron acceptor in the presence of an immobilized enzyme and a substrate for it and are capable of being kept stable in the carrier, particularly in a binder for paste or gel can be used. As preferable electron transfer mediators for oxidases, there can be mentioned p-benzoquinones, ubiquinones, potassium ferricyanide, dichlorophenolindophenol (DCIP), phenazine methosulfates (PMS) and the like. The amount of impregnation is suitably 0.2 - 30 % by weight when p-benzoquinone is used to a graphite paste. A content in a graphite paste of less than 0.2 % by weight is not preferable in respect of current sensitivity, and a content more than 30 % by weight is not preferable in respect of shape maintenance of electrodes and of economical reason. The most preferable content is 15 - 25 % by weight.

As preferable electron transfer mediators for dehydrogenases, there can be mentioned NAD and dichlorophenolindophenol (DCIP). Besides them, however, any compounds which participate in the reaction as an electron acceptor in the presence of a dehydrogenase and a substrate for it and are stable in the carrier, especially in the paste or gel. The amount of impregnation with such electron transfer mediator is suitable 1 - 10 % by weight when NAD is used to a graphite paste. A content in a graphite paste of less than 1 % by weight is not preferably in respect of current sensitivity, and a content more than 10 % by weight is not preferable in respect of shape maintenance of electrodes and of economical reason. The most preferable content is 3 - 5 % by weight.

The enzyme immobilized on the carrier as described above can be selected properly in accordance with the substrate whose detection or conversion is intended. When an oxidase is used, glucose oxidase/glucose, galactose oxidase/galactose, alcohol oxidase/ethanol, cholesterol oxidase/cholesterol, amino acid oxidase/amino acid, uric acid oxidase/uric acid, etc. are representatives of the enzyme/substrate combinations.

As other enzyme/substrate combinations, there can be mentioned alcohol dehydrogenase/ethanol, glucose dehydrogenase/glucose, glutamate dehydrogenase/glutamic acid, lactic acid dehydrogenase/lactic acid, glycerol dehydrogenase/glycerol and the like.

The immobilization of such enzymes on the carrier is performed usually by a solution method, that is, by simply applying or dropping an enzyme solution to or onto a surface of carrier and evaporating the solvent from the solution held on the surface. In this case, it is preferred to make the surface as smooth as possible beforehand. The amount of immobilized enzyme is preferably about 10 - 200 $\mu g/cm^2$ in the case of alcohol dehydrogenase or glucose oxidase. Of course, the immobilization may be effected in any other method (such as chemical binding method), as the case may be.

The substrate-permeable thin film of the present invention serves for holding and fixing the enzyme adhered or fixed onto the surface of carrier and for protecting the enzyme from the externals. As such permeable film to coat at least the portion where enzymes are immobilized, cellulose acetate film, nitrocellulose film,K-carrageenan gel film, polyacrylamide gel film,dialysis film and the like may be used. A nitrocellulose film formed by spreading and drying a solution containing collodion is preferably employed. Although there is no limitation on the thickness of film, it can be generally said that a thinner film is suitable for shortening the response time and a thicker one is favorable for broadening the range of response concentration. Usually, a thickness of about 20 - 500 μm is suitable though it depends on the sort of film.

The internal electrode capable of applying voltage to the enzyme immobilized surface can be a filamentary one which is generally used, or a plane one. For instance, when a porous material, or a paste or gel, of a conductive or semi-conductive substance, is used as the carrier, it is possible to supply voltage to

the enzyme immobilized surface by connecting a filamentary electrode at least to said carrier. When a porous mateiral, or a paste or gel, not using a conductive or semi-conductive substance is employed as the carrier, it is preferred to use a plane electrode, especially a grid electrode having many spaces or of reticular form, and laminate it on the portion of the carrier where enzyme is immobilized, to form an internal electrode. The grid electrode having many spaces is a preferable embodiment, because it is possible by employing it to prevent superfluous permeation or elution onto the carrier surface of the electron transfer mediator in the carrier.

Hereinafter, the present invention is further explained in detail by giving Examples which however shall never constitute any restriction on the invention:

EXAMPLE 1 (A glucose oxidase electrode using glucose oxidase as enzyme and p-benzoquinone as electron transfer mediator)

A) Preparation of electrode

Reagents

Glucose oxidase (GOD)
    Type II, a product of Sigma Co. (EC 1,1,3,4 derived from Aspergillus Niger).
p-Benzoquinone (BQ)
    A product of Wako Pure Chemical Co. (Purified by sublimation before use).
Collodion solution (5%)
    A product of Wako Pure Chemical Co..
Liquid Paraffin
    A product of E. Merck Co..
Graphite powder
    No. ACP, a product of Nippon Kokuen Co..

A prescribed amount of p-benzoquinone was mixed with 3 ml of liquid paraffin, and then 5g of graphite powder was further added to the mixture. The p-benzoquinone/graphite paste thus obtained was packed into one end of a glass tube of 3.4 mm inner diameter. The surface of exposed paste was smoothed by means of a piece of wax-paper to form a paste electrode (the carrier) having a surface area of 9.0×10^{-2} cm^2.

Next, an aqueous GOD solution of a prescribed concentration was dropped by a syringe on the surface of said paste electrode, and then the solvent was evaporated from the solution hold on the surface. Thereafter, 20 µl of a collodion/ethanol (1:4 volume/volume) mixture was spread on the surface and dried to form a thin film of nitrocellulose on the surface of the electrode.

To the electrode (the carrier) thus obtained, an outgoing electrode (the internal electrode) consisting of platinum wire was provided. By further setting a nylon net and a teflon tube for supporting the nitrocellulose film, a glucose oxidase electrode (a GOD-immobilized/benzoquinone-graphite paste electrode) (1) of the present invention as shown in Fig. 1 was prepared. In Fig. 1, (2) denotes the p-benzoquinone/graphite paste electrode (the carrier), (3) denotes the immobilized GOD, (4) denotes the thin nitrocellulose film, (5) denotes the platinum wire, (6) denotes the glass tube, (7) denotes the nylon net and (8) denotes the heat shrinkable teflon tube.

The electrode was washed several times with distilled water and stored overnight in an acetate buffer solution having a pH 5.0 before it was used.

B) Cyclic voltammetry

Voltammetry by three-electrode method was effected using the above-described glucose oxidase electrode. Condition of the measurement were as follows:

Instruments

Potentiostat (made by Fuso Co.)
Signal generator (HB-104, made by Hokuto Electric Co.)
Counter-electrode (platinum plate)
Referential electrode (saturated calomel electrode)
Recorder (X-Y recorder, made by Yokogawa Electric Co.)

Electrolyte

Deoxygenated 0.1 M acetate buffer solution having a pH 5.0
Temperature 23 ± 1 °C
Stirring rate 500 r.p.m.

Results

An electrode of the present invention prepared by immobilizing 18 µg of GOD on a graphite paste electrode containing 0.25% by weight of p-benzoquinone was immersed in the above-described acetate buffer solution and the cyclic voltammogram was recorded at a potential scan rate of 50 mV/s. As shown by the full line in Fig. 2A, the peak potentials were noted at -0.07V and +0.40V (vs SCE) in cathodic and anodic waves, respectively. Although the cyclic potential scan was continued further for several hours, any decrease in the current was not observed. As an electrode similar to this, but not containing p-benzoquinone, was estimated in the same manner as that mentioned above, any voltammetric peak was not observed as shown by the broken line in Fig. 2A.

Thus, it has become apparent that the voltammetric peak are ascribed to the electrochemical redox reaction of p-benzoquinone which has moved from the paste to the interfacial space between the graphite paste electrode (the carrier) and the thin nitrocellulose film, and the p-benzoquinone molecules contribute to the electrochemical reduction reaction.

Although p-benzoquinone might leak out through the thin nitrocellulose film slowly into the electrolyte, p-benzoquinone is supplied from the graphite paste medium so that the concentration of p-benzoquinone at the interface between the graphite paste and the thin nitrocellulose film is kept substantially constant.

C) Electrocatalytic oxidation of glucose

When glucose was added to the above-described buffer solution, it was observed that the anodic current of the electrode of the present invention was significantly increased as shown by the full line in Fig. 2B, and that the steady-state current Is at a given potential increased as the concentration of glucose in the solution measured was increased, as shown in the Figs. 3A and 3B. It was found that the current Is increased with the increasing content of p-benzoquinone in the graphite paste within the range of 0.25 - 10% by weight and it approached almost a limiting value as the content exceeded 10% by weight. The current Is depended also on the amount of GOD applied on the surface of the carrier. For example, paste electrodes containing 20% by weight of p-benzoquinone on which 1.8 µg and 18 µg of GOD were immobilized produced the currents Is of 2 µA and 32 µA, respectively.

These results indicate that GOD immobilized on the surface of electrodes maintains activity and that p-benzoquinone entrapped at the interface acts as an electron transfer mediator between the immobilized GOD and the paste electrode.

As to a p-benzoquinone (30% by weight)/graphite paste electrode on which 18 µg of GOD is immobilized, the dependency of Is at an impressed voltage of 0.5V on the glucose concentration (Cglu) is shown in Figs. 3A and 3B. The maximum current Is^{max} and the apparent Michaelis constant were determined to be 124 µA and 104 mM, respectively, from the intercept and the slope of the double reciprocal plot of the data in Figs. 3A and 3B. From this Is^{max} value, it is estimated that the amount of electrocatalytically active GOD immobilized on the carrier is 3×10^{-11} mol/cm² which corresponds to about 3% of the total amount of GOD used, as the catalytic activity were assumed to be the same for both immobilized GOD molecules and GOD molecules dissolved in the solution measured.

The above electrode showed a linear current response to the glucose concentration, up to the concentration of 15 mM, with a correlation coefficient of 0.9999. Since the background current was very small (0.2 ± 0.01 µA), it was possible to determine glucose concentrations lower than 1 mM with a coefficient of variation of 5.3% (n=5) at Cglu = 1mM (reference is made to Fig. 3B). The response of current was fast and the steady-state current was reached within 20 seconds.

As the stability of the electrode was evaluted by periodically testing its response to 8.3 mM gluocse, it was found that its original activity was retained more than a weak, the electrode being stored in the buffer solution at temperatures lower than 5 °C when not in use.

As the reproducibility of preparing the electrode was evaluated by the response to 8.3 mM glucose, with regard to four sensors prepared by immobilizing 18 µg of GOD on p-benzoquinone (30% by weight)-/graphite paste electrodes, they gave an average Is value of 7.9 µA with a standered deviation of 0.7 µA.

In order to evaluate the influence of oxygen, Is of air-saturated solution and Is of deaerated solution

were measured with respect to solutions containing 5 - 40 mM of glucose. The results are shown in Fig. 4. It was found that the influence was small over the studied range of glucose concentration, which covered the glucose concentrations usually detected in blood samples.

The electrodes of the present invention work suitably in solutions having a pH within the range 5 to 8.

EXAMPLE 2

A glucose oxidase electrode was prepared in the same manner as Example 1, except that potassium ferricyanide was used instead of p-benzoquinone as the electron acceptor.

With this electrode, the same cyclic voltammetry was effected in the same manner as Example 1. As shown in Fig. 5, voltammetric peaks were observed. Thus, it was made apparent that potassium ferricyanide contributed to the electrochemical reaction.

EXAMPLE 3

An electrode of such constitution as the counter-electrode was set inside the glass tube (1) was prepared accroding to the manner as described in Example 1 (A). The constitution of the electrode is shown in Fig. 6, wherein (10) denotes a silver/silver chloride electrode constituting the counter-electrode (cathode), which winds around the inner supporting material (6') and is fixed thereon, (9) denotes the solution inside the electrode, consisting of acetate buffer solution containing potassium chloride, and other numerical figures correspond to those described before.

EXAMPLE 4 (Alcohol dehydrogenase electrode)

An alcohol dehydrogenase electrode of the present invention was prepared in the same manner as Example 1 (A), except that alcohol dehydrogenase (ADH) (EC 1,1,3,4: a product of Sigma Co.) was used instead of GOD and nicotinamide adenine dinucleotide (a product of Sigma Co.) was used instead of BQ.

With this electrode, the cyclic voltammetry was effected in the same manner as Example 1 (B).

Electrolyte

Deoxygenated tris-hydrochloride buffer solution having a pH 8.3

Temperature	23 ± 1 °C
Stirring rate	500 r.p.m.

Results

An electrode of the present invention prepared by immobilizing 20 µg of ADH on a graphite paste electrode containing 3% by weight of NAD was immersed in the above-mentioned tris-hydrochloride buffer solution and a cyclic voltammogram was recorded at a potential scan rate of 50 m V/s. The results obtained are shown by the full line in Fig. 7.

The results were same as the voltammogram recorded in the same manner with respect to an electrode similar to the above one, but not containing NAD.

On the other hand, it was found that an electrode containing NADH, i.e. a reduced type NAD, gave an anodic wave having a peak at + 0.6V as shown by the broken line in Fig. 1. Thus, it was found that only NADH, which is a reduced type of NAD, showed electrochemical response at a carbon paste electrode. That is, it was found that NADH was irreversibly oxidized electrochemically at a paste electrode, according to the equation:

$$\mathrm{NADH} \xrightarrow{-2e} \mathrm{NAD}^+$$

and eventually NAD could be reproduced at the present electrode.

Electrochemical catalytic oxidation of ethanol

As ethanol was added to the above-mentioned buffer solution, an increase of anodic current was noted at a positive potential higher than + 0.6V. It was found that, with increasing the ethanol concentration in the solution measured, the steady-state current Is increased. It was found that the steady-state current increased also depending on the amount of NAD in the graphite paste and on the amount of ADH on the surface of the electrode.

These results indicate that the immobilized ADH retains its activity and so the NAD entrapped at the interface between the immobilized ADH and the paste electrode acts as electron acceptor in the enzyme reaction of ADH and is reduced to NADH, and that NAD is reproduced electrochemically from such NADH and as a result the steady-state current appears.

The dependency to ethanol concentration of Is at an impressed voltage of 0.8V (against a saturated calomel electrode) of an electrode prepared by immobilizing 20 μg of ADH on a NAD (3% by weight)-/graphite paste is shown in Fig. 8. This figure can be used as a calibration curve on measuring by means of the present electrode the ethanol concentration in the solution measured. The present electrode shows a linear response, at an ethanol concentration lower than 10 mM. The response of current is fast, and the steady-state current value is reached within 20 seconds.

EXAMPLE 5 (Glucose dehydrogenase electrode)

A glucose dehydrogenase electrode same as that shown in Fig. 4 was prepared in the same manner as Example 1(A), except that glucose dehydrogenase (GDH: a crude extract specimen isolated from Pseudomonas fluorescens) was used instead of ADH in Example 4 and dichiorophenolindophenol (DCIP:a product of Nakarai Chemical Co.) was used instead of NAD. The electrode was washed several times with distilled water and immersed and stored overnight in an acetate buffer solution of pH 5.0, before it was used.

Voltammetry by three-electrode method was effected using this electrode in the same manner as Exampel 1(B). That is, an electrode of the present invention prepared by immobilizing 100 μg of GDH on a graphite paste electrode containing 3% by weight of DCIP was immersed in an acetate buffer solution having a pH 5.0 and the cyclic voltammogram was recorded at a potential scan rate of 50 m V/s. The results are shown in Fig. 9. The peak was noted at -0.2V for cathodic wave, and at +0.3V for anodic wave.

When glucose was added to the above buffer solution, an increase of anodic current was observed at a positive potential higher than +0.4V. It was found that the steady-state current Is increased as the glucose concentration in the solution measured was increased. Further, it was also found that the current Is increased in dependence on the content of DCIP in the graphite paste electrode and the amount of GDH on the surface of electrode.

The characteristic response at this electrode to glucose concentrations in the solution emasured is shown in Fig. 10. It was found that the anodic current at +0.5V began to increase when 10 mM glucose was added to the solution measured (the arrow in the figure) and the steady-state current was reached after about 1 minute.

EXAMPLE 6

A GOD electrode was prepared using gold powder instead of the graphite powder used in Example 1.

At first 0.1 ml of liquid paraffin was added to 0,1g of gold powder, the mixture was well mixed in an agate mortor, and then 40 mg of p-benzoquinone was further added to the mixture and the resulting mixture was well mixed. The p-benzoquinone/gold paste thus obtained was packed into one end of a glass tube having an inner diameter of 3.4 mm, and the surface of the paste was smoothed with a piece of wax paper.

A GOD electrode same as that shown in Fig. was prepared in the same manner as Example 1.

This electrode was immersed in an acetate buffer solution of a pH 5.0, and the cyclic voltammogram was recorded at a potential scan rate of 50m V/S. As shown by the full line in Fig. 11, the peak in cathodic and anodic waves was observed at -0.07V and +0.40V (vs SCE), respectively. Any decrease of current was not observed even when the cyclic potential scan was continued further for several hours. Although the same evaluation as above was effected with regard to an electrode similar to the above one, but not containing p-benzoquinone, any voltammetric peak was not observed as shown by the broken line in Fig. 11.

Electrocatalytic oxidation of glucose

By setting a positive potential well higher than the redox potential of p-benzoquinone (E = +0.5V vs.

Ag/ AgCl) and adding glucose successively to the above buffer solution to increase the glucose concentration (0 → 200 mM), the increase of the current from the base was measured. The results are shown in Fig. 12.

From the results, it was found that the electrode of the present invention could correspond even to a minute variation in glucose concentration and so was useful as an electrode for measuring glucose.

The same results as above were obtained also when powder of other metal (such as platinum), semiconductor (such as titanium oxide, tin oxide, etc.) or high molecular conductor (such as polypyrrole, polyacetylene, etc.) was used instead of the aobve gold powder.

EXAMPLE 7

A porous graphite plate (RVC 2 x 1 x 100 mm, INF, a product of Chemotronix Co.) was used as the carrier.

First, a cylinder having a diameter of 3.4 mm was cut out by a cork borer from the porous graphite plate and a lead wire was connected to one end of the cylinder with a conductive bonding agent. To prevent immersion of solvent, an epoxy bonding agent was applied to the whole surface where the lead wire was adhered, and at the same time said surface was adhered to a glass tube having an inner diameter of 3.4 mm. Then, insulation of the side surface of electrode and fixation of electrode were effected by means of a heat shrinkable tube. The pores of the porous electrode thus prepared were impregnated with a quinone-containing material to form a storage layer (the carrier of the present invention). The quinone-containing material was prepared by mixing 40 mg of p-benzoquinone with 0.3 ml of liquid paraffin in a mortar.

Next, 10 µl of GOD (1 mg/ml) was supplied onto the surface of the storage layer and then water was evaporated. Thereafter, the surface was covered with nylon net, and 20 µl of a collodion/ethanol (1/4 V/V) mixture was spread on the surface and dried to form a thin nitrocellulose film on the surface of electrode. The nylon net was fixed to the electrode with parafilm, while applying grease to the nylon net at the portion not covering the surface of electrode.

The electrode (100) for measuring glucose, obtained as described above, is shown in Fig. 13. In this figure, (22) denotes the storage layer, (23) the immobilized GOD layer, (24) the thin nitrocellulose layer, (25) the lead wire (platinum wire), (26) the glass tube, (27) the nylon net, (28) the heat shrinkable tube, (29) the conductive bonding agent and (20) the epoxy resin, respectively.

The electrode (100) was washed several times with distilled water and stored overnight in an acetate buffer solution of pH 5.0, before it was used.

The electrode was immersed in the above-described acetate buffer solution and the cyclic voltammogram was recorded at a potential scan rate of 50m V/s, in the same manner as described above. Peaks in cathodic and anodic waves were observed at -0.07V and +0.40V (vs SCE), respectively, as shown by the full line in Fig. 14. Any decrease of current was not observed as the cyclic potential scan was continued further for several hours. As a similar electrode not containing p-benzoquinone was evaluated in the same manner as above, any voltammetric peak was not observed as shown by the broken line in Fig. 14.

Electrocatalytic oxidation of glucose

By setting a positive potential well higher than the redox potential of p-benzoquinone (E = +0.5V vs. Ag/AgCl) and adding glucose successively to the above buffer solution to increase its concentration (0 → 100 mM), the increase of the current from the base was measured. The results are shown in Fig. 15.

From the results, it was found that the electrode of the present invention could correspond even to a minute variation in glucose concentrations and so was useful as an electrode for measuring glucose.

EXAMPLE 8

GOD electrodes were prepared in the same manner as Example 7, using the following materials instead of the quinone-containing material in Example 7, and their responsibility to glucose was evaluated.

The results are shown in the following table:

Table

Storage layer (impregnating graphite)	p-Benzoquinone (mg)	Dispersant	(ml)	Agent for mixing paste	(mg)	Base E=0,5V (μA)	Response to 20mM glucose (μA)
Gel containing quinone dissolved	40	Liquid paraffin	0.3	Gelol D	25	72	128
Quinone teflon paste	20	® Valflon paste			200	2.35	0.60
Quinone silicone grease	10	Silicone grease	100	-		0.19	0.06
Quinone nujol cellulose	20	Liquid paraffin	0.15	Cellulose powder	100	0.32	1.33
Quinone vaseline	20	White vaseline	200 mg	-		0.09	1.28
Quinone cemedine	40	® Cemedine-C	400 mg	-		48.5	2.0
Quinone nujol carbon powder	40	Liquid paraffin	0.3	Carbon	500	0.30	31.20

EXAMPLE 9

A GOD electrode was prepared by using a mini-grid electrode as the internal electrode.

Electrode material

Gold mini-grid electrode No. MG-42, 500 wires/in. a product of Buckbee-Mears. Co.

At first, 0.3 ml of liquid paraffin was added to 500 mg of graphite powder and mixed in a mortar, and then 40 mg of p-benzoquinone was added to and uniformly mixed with the resulting mixture. The mixture so obtained was packed into a glass tube having an inner diameter of 3.4 mm and the surface was smoothed with a piece of wax paper to form the storage layer (the carrier). Next, 10 μl of GOD solution was placed on the storage layer and the solvent was removed by evaporation, and then the above gold mini-grid electrode was placed and fixed by nylon net on the resulting storage layer. Further, 10 μl of GOD solution (1 mg/ml) was placed on the gold mini-grid electrode through the nylon net and the solvent was made to evaporate. Then, after 20 μl of a collodion/ethanol solution (1:4) was placed on the electrode and dried, the mini-grid electrode was connected with the lead wire by means of a conductive bonding agent to give an electrode (110) shown in Fig. 16.

Numeral figures are common to Fig. 16 and Fig. 13. In Fig. 16, (32) denotes the storage layer, (13) denotes the gold mini-grid electrode and (14) denotes the collodion film.

The electrode (110) was stored in the same manner as Example 1 until it was used.

Cyclic voltammetry

As the cyclic voltammetry was effected under the same measuring conditions as Example 7, the results shown in Fig. 17 were obtained. As shown in Fig. 17, it was found that, also in the electrode of this Example, p-benzoquinone moving from the storage layer acted as the electron transfer mediator.

Electrocatalytic oxidation of glucose

In the same manner as Example 7, the increase of the current from the base was measured by adding glucose successively to the buffer solution to increase its concentration.

The results are shown in Fig. 18. It was found from the results that the above electrode could correspond even to a minute variation in glucose concentrations and so was useful as an electrode for measuring glucose.

Also when a mini-grid electrode was used, the same results as above were obtained with respect to various storage layers mentioned in Example 6. The mini-grid electrode could be a metal mini-grid electrode other than the gold mini-grid electrode, such as a platinum mini-grid electrode. It was suitable to compose the mini-grid electrode with a noble metal.

EXAMPLE 10

In a mortar, 100 mg of cellulose powder, 200 μl of liquid paraffin (nyjol) and 48 mg of benzoquinone were mixed together. The resulting mixture was packed into one end of a glass tube having an inner diameter of 3.4 mm and an outer diameter of 5 mm, and the surface was smoothed by means of a piece of paraffin paper. On the surface of the paste carrier, a platinum net electrode (100 mesh) cut 5 mm square was placed and a lead was connected to a part of it with a conductive bonding agent. Then, the net portion protruding from the glass tube was turned up and fixed by shielding with an epoxy bonding agent. On the carrier surface where the net electrode was laminated, 5 μl of GOD solution (100 mg/ml) was placed. The surface was covered further with a dialysis film (0.025 mm, a product of Visking Co.) and the dialysis film was fixed with O-ring, whereby an enzyme electrode (120) of the present invention as shown in Fig. 19 was obtained. In Fig. 19, (13a) denotes the platinum net electrode, (23) denotes the immobilized GOD layer, (25) the lead wire, (26) the glass tube, (29) the epoxy bonding agent, (34) the dialysis film, (40) the O-ring, and (42) the paste carrier, respectively.

After leaving the electrode to stand overnight, measurement was effected. At first, the cyclic voltammogram was recorded in a deoxygenated sodium phosphate buffer solution having a pH of 7.0, using a silver/silver chloride electrode as the reference electrode. As shown in Fig. 20, the reduction wave of benzoquinone was observed at -80 mV and the oxidation wave of hydroquinone at +300 mV.

Next, by fixing potential at E=450 mV (vs Ag/AgCl) and adding glucose to the above deoxygenated sodium phosphate buffer solution (25 °C) having a pH of 7.0 under nitrogen stream, a catalytic increase of current was confirmed. The relation between the increase of current and the concentration of glucose added is shown in Fig. 21. Thus, it was found that estimation could be effected upto a high concentration of glucose.

The enzyme electrode of the present invention shows a high current response to the substrate such as glucose, ethanol or the like, without any other electron transfer mediator added into the solution measured.

Therefore, the electrode can suitably be used as sensor for various substrates. Further, it has an effect that it is not affected substantially by any variation in oxygen portial pressure even when oxygen is present. Moreover, the ceiling phenomenon of measured values at high concentration region, which has been observed in the oxygen electrodes hithertofore used for measurement of glucose and which results from shortage of oxygen, can be solved by the electrode of the present invention.

Further, the enzyme electrode of the present invention has a merit that the electron transfer mediator is hardly consumed by reaction since the electron transfer mediator reduced by oxidation reaction is regenerated (re-oxidized) by electrode reaction.

In addition, the electrode of the present invention can maintain the high enzyme reactivity of dehydrogenases almost constant over a long period (usually, more than one week), because a graphite paste electrode containing a larger amount of electron accepting compounds such as NAD as compared with hithertofore used NAD immobilized dehydrogenase electrodes and being capable of supplying said compounds always to the enzyme immobilized layer is used.

Claims

1. An enzyme electrode comprising a carrier, an enzyme immobilized on a part of the outer surface of the carrier, a coating film consisting of a thin film permeable for a substrate for the enzyme and coating the portion where the enzyme is immobilized, and an internal electrode capable of applying voltage to the portion,
said carrier being in the form of a porous material, paste or gel being mixed with a substance acting as electron transfer mediator to provide a storage layer for the mediator whereby the concentration of the mediator at said portion is able to keep substantially constant for a long period.

2. An enzyme electrode as claimed in claim 1, wherein said porous material, paste or gel contains a conductive or semi-conductive substance.

3. An enzyme electrode as claimed in claim 2, wherein said conductive or semi-conductive substance is graphite, a noble metal, a high molecular conductor or an inorganic semi-conductor.

4. An enzyme electrode as claimed in claim 1, wherein the substance which acts as an electron transfer mediator is selected from the group consisting of p-benzoquinones, ubiquinones, potassium ferricyanide, dichlorphenolindophenol (DCIP), phenazine methosulfates (PMS) and nicotinamide adenine dinucleotide (NAD).

5. An enzyme electrode as claimed in claim 1, wherein the carrier is in a paste form in which p-benzoquinone as an electron transfer mediator is admixed at 0.2 - 30 % by weight.

6. An enzyme electrode as claimed in claim 1, wherein the carrier is in a paste form in which NAD as an electron transfer mediator is admixed at 1 - 10 % by weight.

7. An enzyme electrode as claimed in claim 1, wherein the enzyme immobilized is an oxidase such as glucose oxidase, galactose oxidase, alcohol oxidase, or a dehydrogenase such as alcohol dehydrogenase, glucose dehydrogenase, glutamate dehydrogenase, lactic acid dehydrogenase or glycerol dehydrogenase.

8. An enzyme electrode as claimed in claim 1, wherein the thin film permeable for the substrate is selected from the group consisting of cellulose acetate film, nitrocellulose film, K-carrageenan gel film and polyacrylamide gel film.

9. An enzyme electrode as claimed in claim 1, wherein the internal electrode is a filamentary or grid electrode.

10. Method for measuring a substrate such as glucose, galactose, ethanol, cholesterol, amino acid, glutamic acid, lactic acid or glycerol using the enzyme electrode of claim 1.

Revendications

1. Electrode enzymatique comportant un support, une enzyme immobilisée sur une partie de la surface extérieure du support, une couche de revêtement formée d'une mince couche perméable pour un substrat destiné à l'enzyme et recouvrant la portion où l'enzyme est immobilisée, et une électrode interne capable d'appliquer une tension à ladite portion, ledit support se présentant sous forme de matériau poreux, de pâte ou de gel, mélangé à une substance agissant comme médiateur de transfert d'électrons, de façon à fournir une couche de stockage au médiateur, la concentration du médiateur au niveau de la portion étant capable de rester sensiblement constante pendant une longue durée.

2. Electrode enzymatique selon la revendication 1, dans laquelle ledit matériau poreux, la pâte ou le gel, contient une substance conductrice ou semi-conductrice.

3. Electrode enzymatique selon la revendication 2, dans laquelle ladite substance conductrice ou semi-conductrice est du graphite, un métal précieux, un conducteur de poids moléculaire élevé ou un semi-conducteur inorganique.

4. Electrode enzymatique selon la revendication 1, dans laquelle la substance qui agit comme médiateur de transfert d'électrons est choisie dans le groupe comprenant les p-benzoquinones, les ubiquinones, le ferricyanure de potassium, le dichlorophénolindophénol (DCIP), les phénazine-méthosulfates (PMS) et le nicotinamide adénine-dinucléotide (NAD).

5. Electrode enzymatique selon la revendication 1, dans laquelle le support se présente sous la forme d'une pâte dans laquelle de la p-benzoquinone, servant de médiateur de transfert d'électrons, est ajoutée à raison de 0,2 à 30 % en poids.

6. Electrode enzymatique selon la revendication 1, dans laquelle le support se présente sous la forme d'une pâte dans laquelle du NAD, servant de médiateur de transfert d'électrons, est ajouté à raison de 1 à 10 % en poids.

7. Electrode enzymatique selon la revendication 1, dans laquelle l'enzyme immobilisée est une oxydase, comme la glucose-oxydase, la galactose-oxydase, l'alcool-oxydase, ou une déshydrogénase, comme l'alcool-déshydrogénase, la glucose-déshydrogénase, la glutamate-déshydrogénase, l'acide lactique-déshydrogénase ou la glycérol-déshydrogénase.

8. Electrode enzymatique selon la revendication 1, dans laquelle la mince couche perméable pour le substrat est choisie dans le groupe comprenant une couche d'acétate de cellulose, une couche de nitrocellulose, une couche de gel de K-carragheen et une couche de gel de polyacrylamide.

9. Electrode enzymatique selon la revendication 1, dans laquelle l'électrode interne est une électrode à filaments ou à grille.

10. Procédé pour mesurer un substrat comme le glucose, le galactose, l'éthanol, le cholestérol, un acide aminé, l'acide glutamique, l'acide lactique ou le glycérol, au moyen de l'électrode enzymatique selon la revendication 1.

Patentansprüche

1. Enzymelektrode, enthaltend einen Träger, ein Enzym, das unbeweglich auf einem Teil der äußeren Oberfläche des Trägers sitzt, einen Beschichtungsfilm, bestehend aus einem dünnen Film, der für ein Substrat für das Enzym durchlässig ist und den Teil beschichtet, an dem das Enzym festgelegt ist, und eine innere Elektrode, die in der Lage ist, dem Teil Spannung zuzuführen, wobei der Träger in Form eines porösen Materials, einer Paste oder eines Gels vorliegt, das mit einer Substanz gemischt ist, die als Elektronenübertragungsbeschleuniger dient, um eine Speicherschicht für den Beschleuniger herzustellen, wodurch die Konzentration des Beschleunigers an diesem Teil in der Lage ist, im wesentlichen für eine längere Zeitdauer konstant zu bleiben.

2. Enzymelektrode nach Anspruch 1, wobei das poröse Material, die Paste oder das Gel eine leitende oder halbleitende Substanz enthält.

3. Enzymelektrode nach Anspruch 2, wobei die leitende oder halbleitence Substanz Graphit, ein Edelmetall, ein Hochmolekularleiter oder ein anorganischer Halbleiter ist.

4. Enzymelektrode nach Anspruch 1, wobei die Substanz, die als Elektonenübertragungsbeschleuniger dient aus der Gruppe ausgewählt ist, die p-Benzochinone, Ubichinone, Kaliumferricyanid, Dichlorphenolindophenol (DCIP), Phenazinmethosulfate (PMS) und Nikotinamid-Adenindinucleotide (NAD) enthält.

5. Enzymelektrode nach Anspruch 1, wobei der Träger in Pastenform vorliegt, bei der p-Benzochinon als Elektronenübertragungsbeschleuniger mit einem Gewicht von 0,2 - 30% beigement ist.

6. Enzymelektrode nach Anspruch 1, wobei der Träger in Pastenform vorliegt, bei der NAD als Elektronenübertragungsbeschleuniger mit einem Gewicht von 1 - 10% beigement ist.

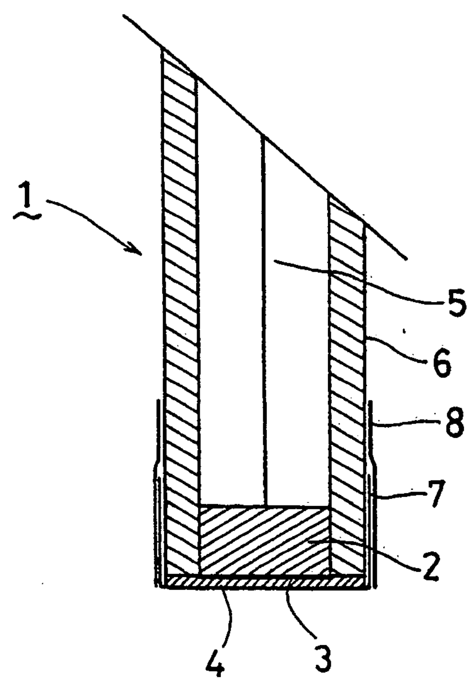
7. Enzymelektrode nach Anspruch 1, wobei das unbewegliche Enzym eine Oxidase ist, wie Glukose-Oxidase, Galaktose-Oxidase, Alkohol-Oxidase, oder ein Dehydrogenase, wie Alkoholdehydrogenase, Glukose-Dehydrogenase Glutamatdehydrogenase, Milchsäuredehydrogenase oder Glyceroldehydrogenase.

8. Enzymelektrode nach Anspruch 1, wobei der dünne Film, der für das Substrat durchlässig ist, aus der Gruppe ausgewählt ist, die Celluloseacetatfilm, Nitrocellulosefilm, K-Karrageen-Gelfilm und Polyacrylamidgelfilm enthält.

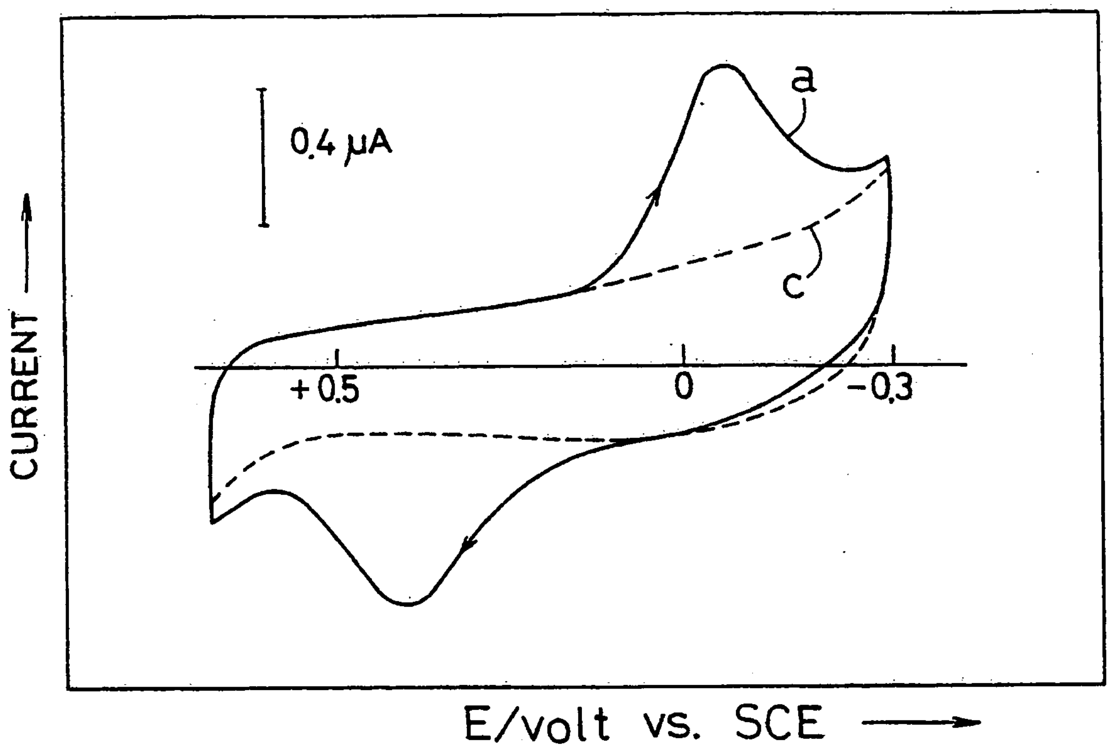
9. Enzymelektrode nach Anspruch 1, wobei die innere Elektrode eine faserförmige oder Gitterelektrode ist.

10. Verfahren zum Messen eines Substrates, wie Glukose, Galaktose, Ethanol, Cholesterol, Aminosäure, Glutaminsäure, Milchsäure oder Glycerol unter Verwendung der Enzymelektrode nach Anspruch 1.

FIG. 1



F I G. 2A


0.4 μA
a
c
+0.5
0
-0.3
CURRENT
E/volt vs. SCE

F I G. 2B

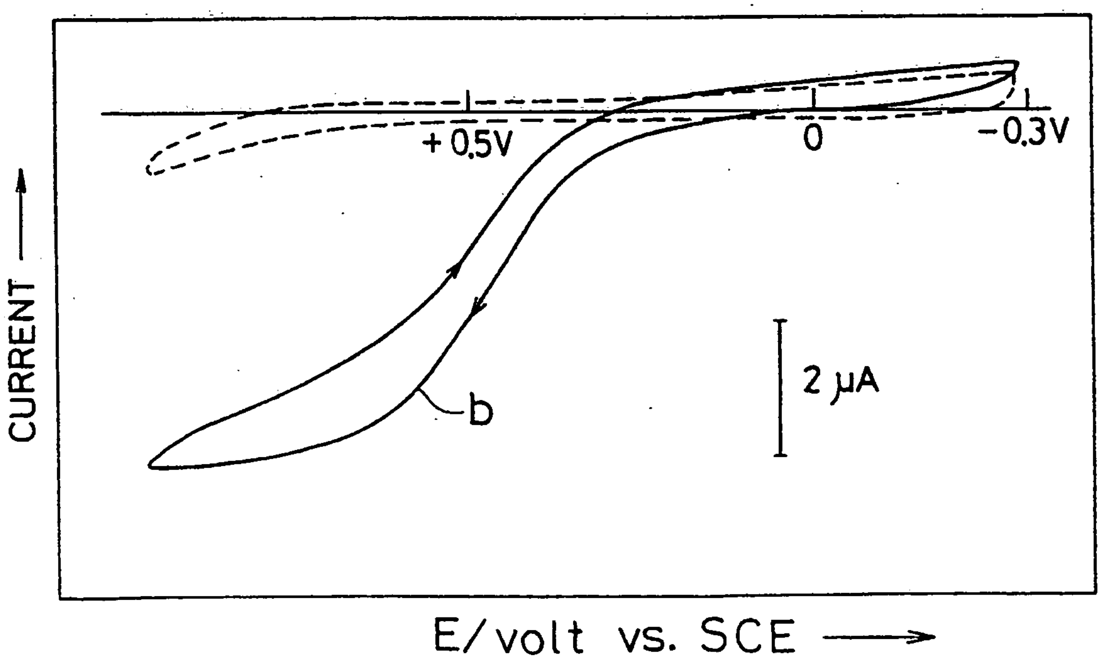

+0.5V
0
-0.3V
2 μA
b
CURRENT
E/volt vs. SCE

FIG. 3A

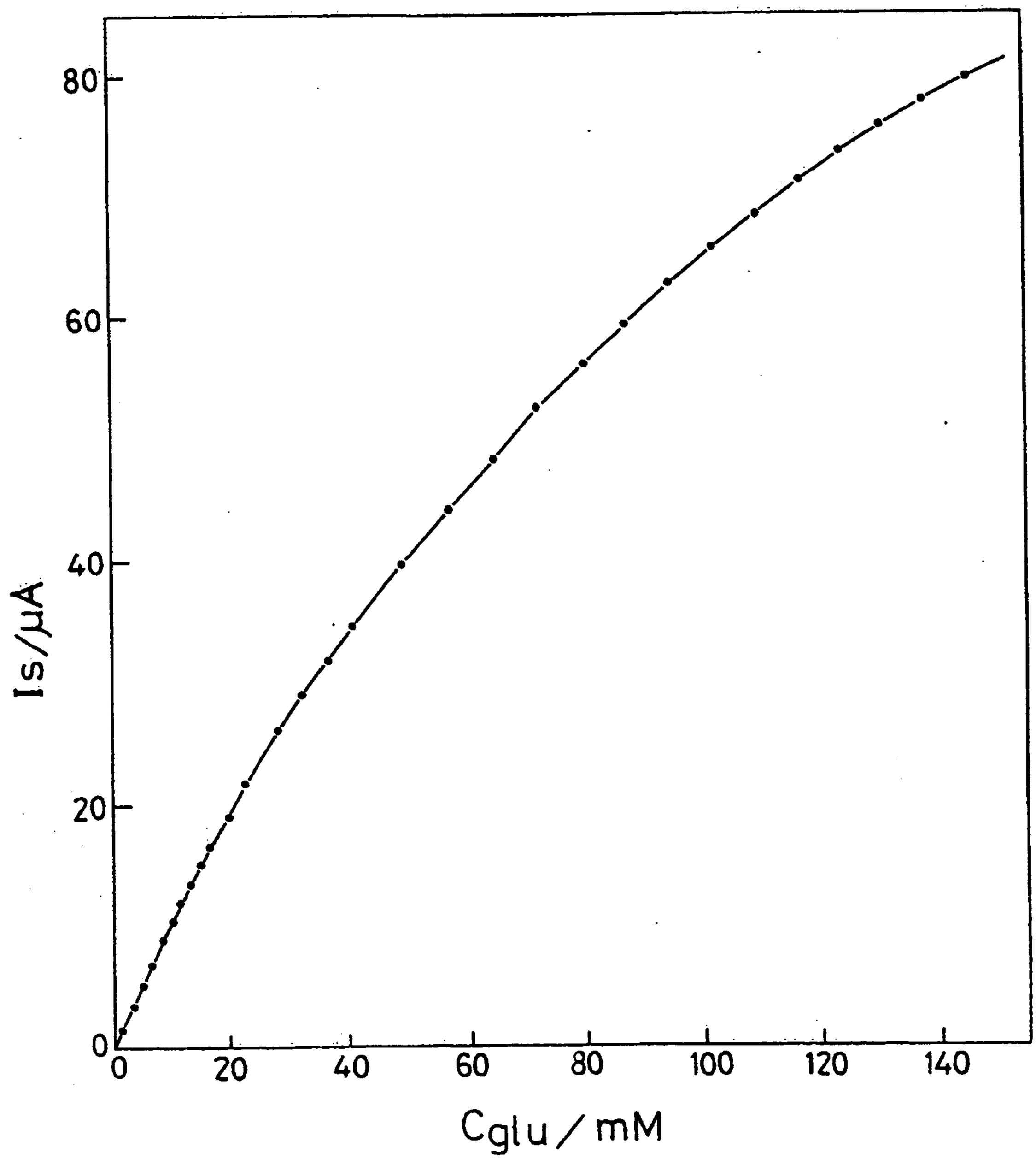


FIG. 3B

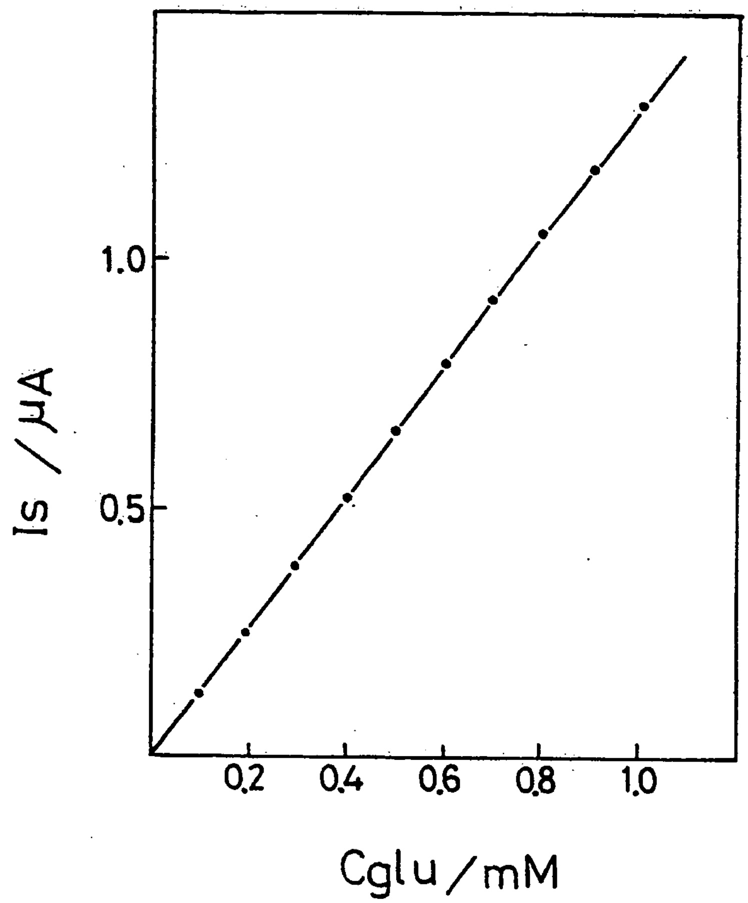


FIG. 4

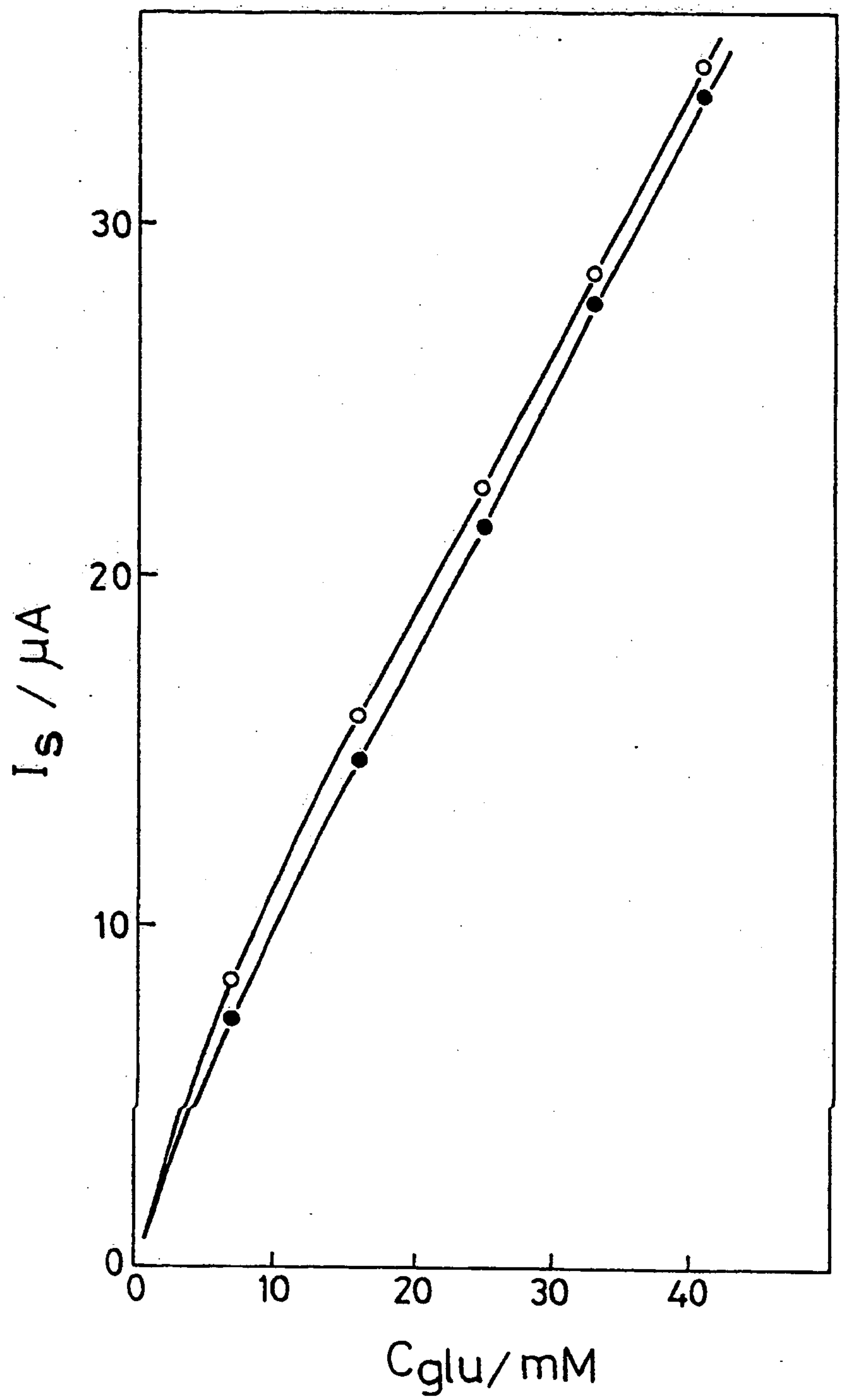


FIG. 5

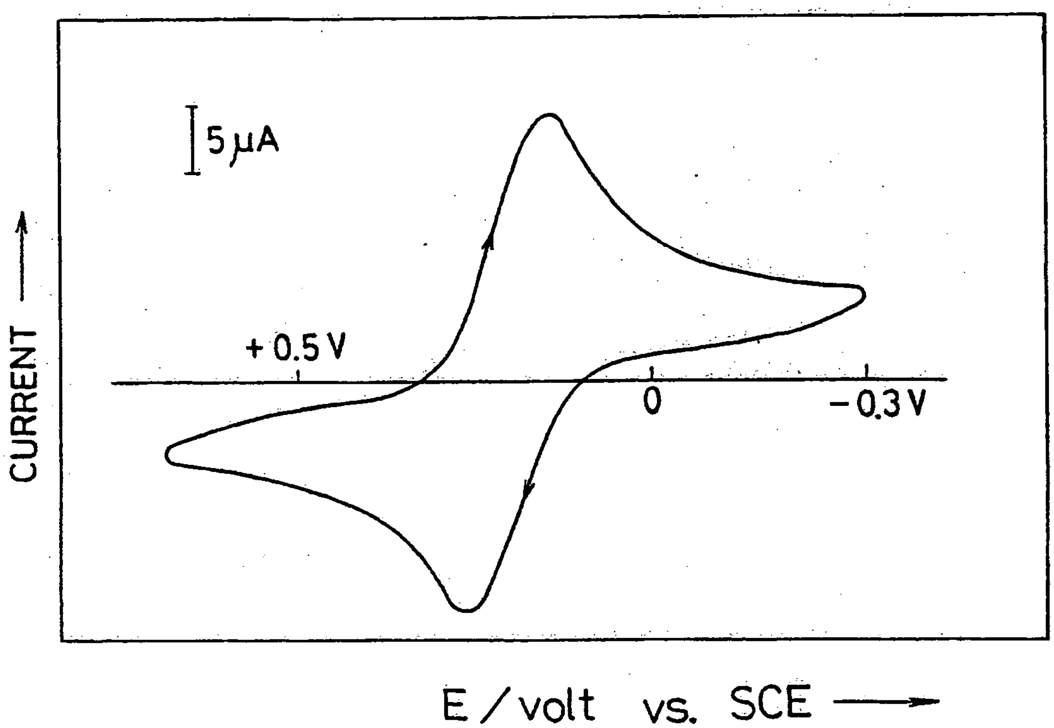


FIG. 6

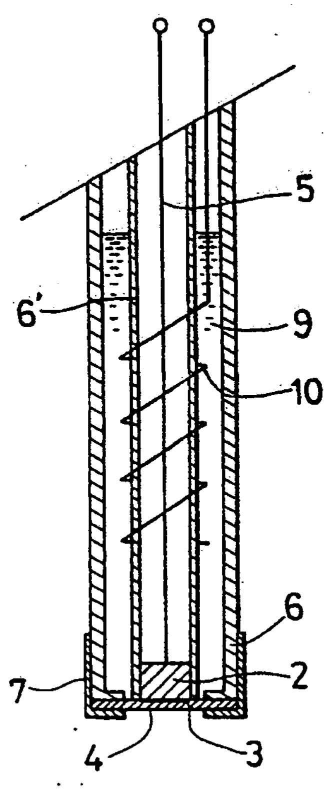


FIG. 7

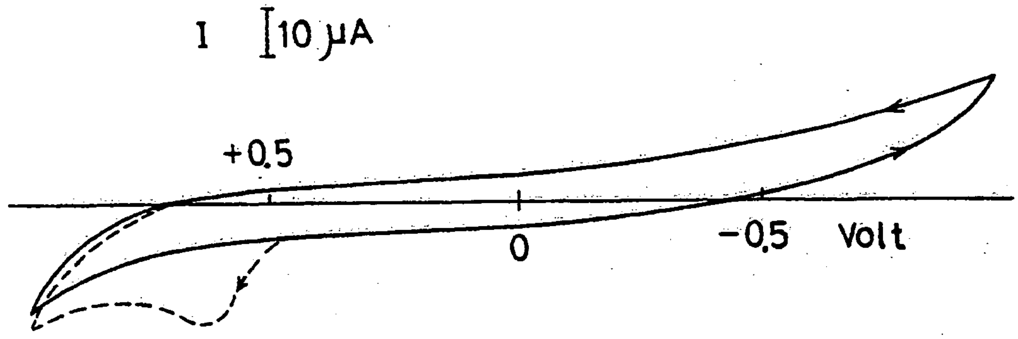


FIG. 8

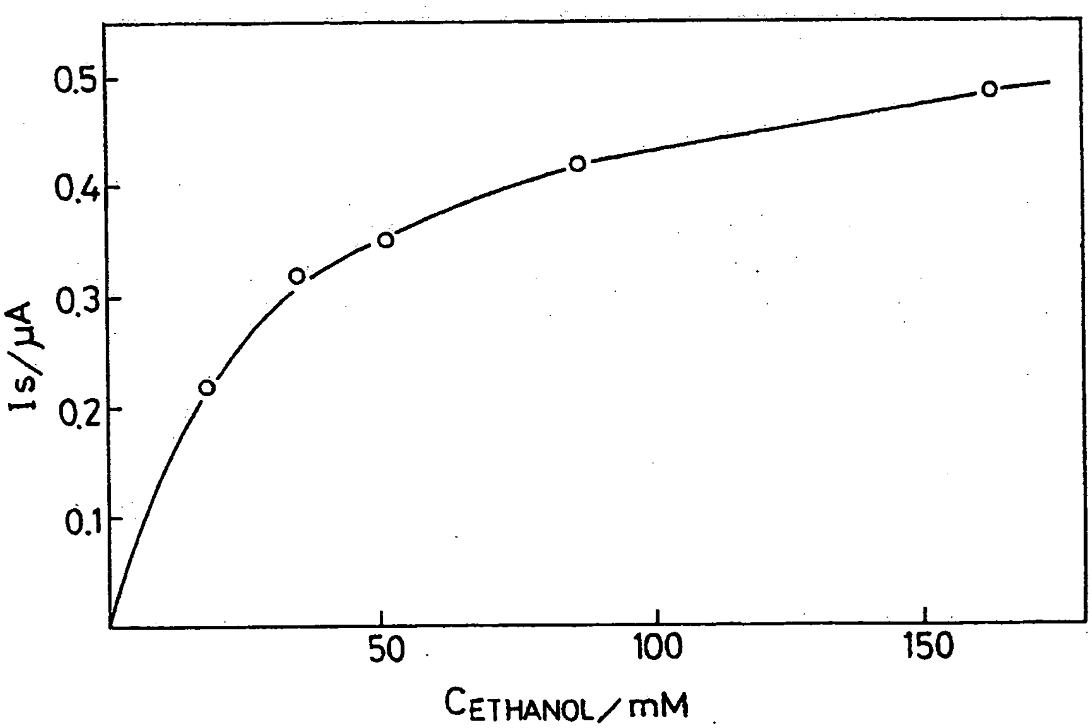


FIG. 9

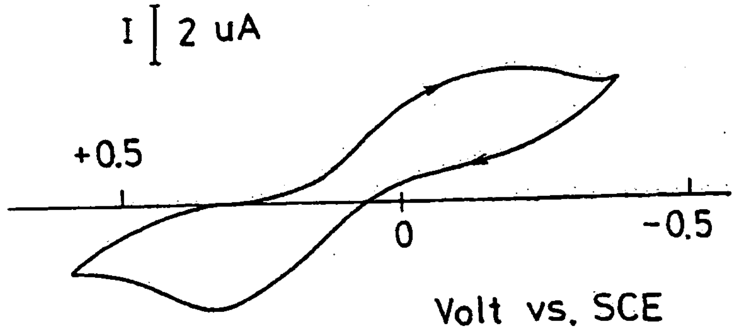


FIG. 10

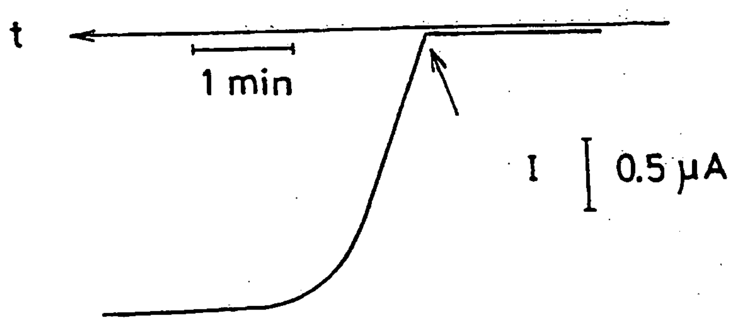


FIG. 11

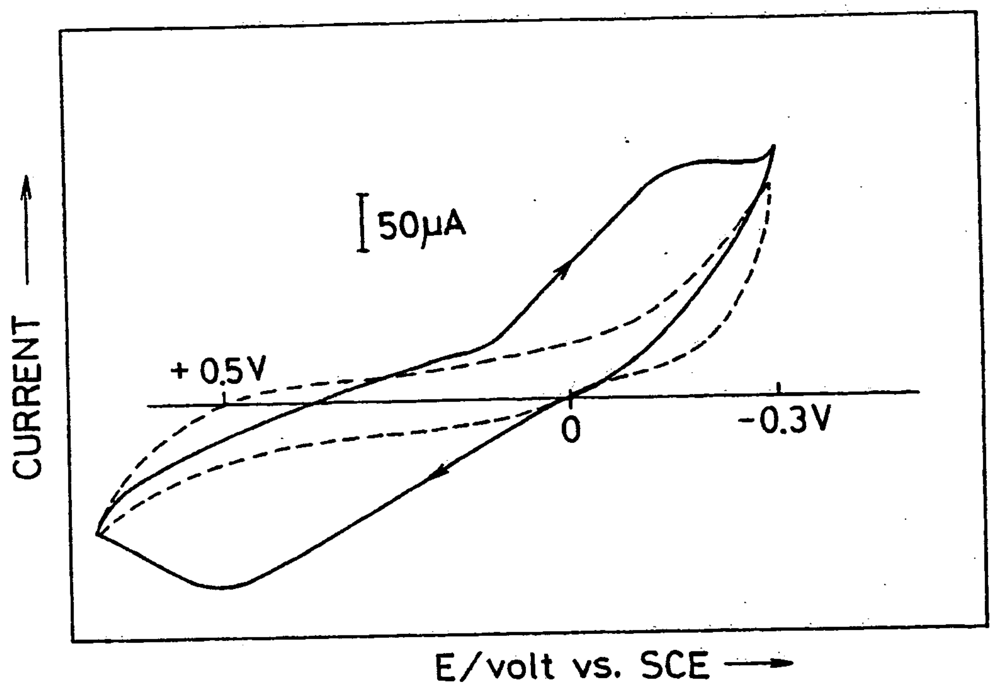


FIG. 12

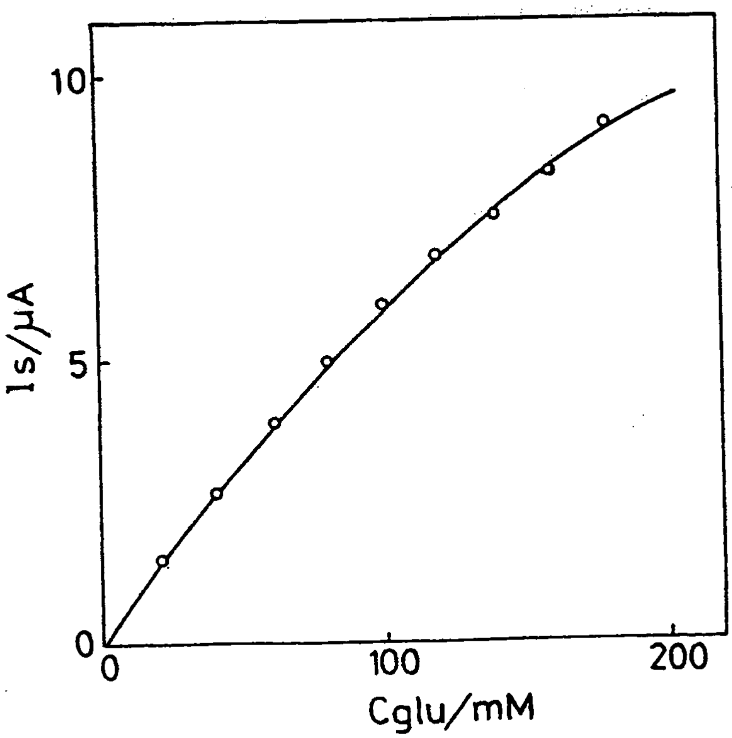


FIG. 13

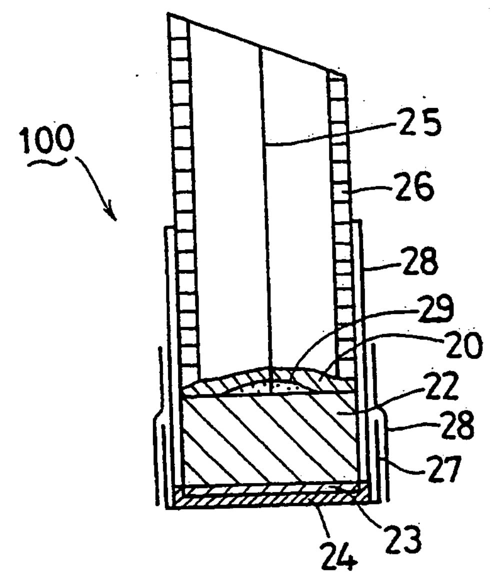

100
25
26
28
29
20
22
28
27
23
24

FIG. 14

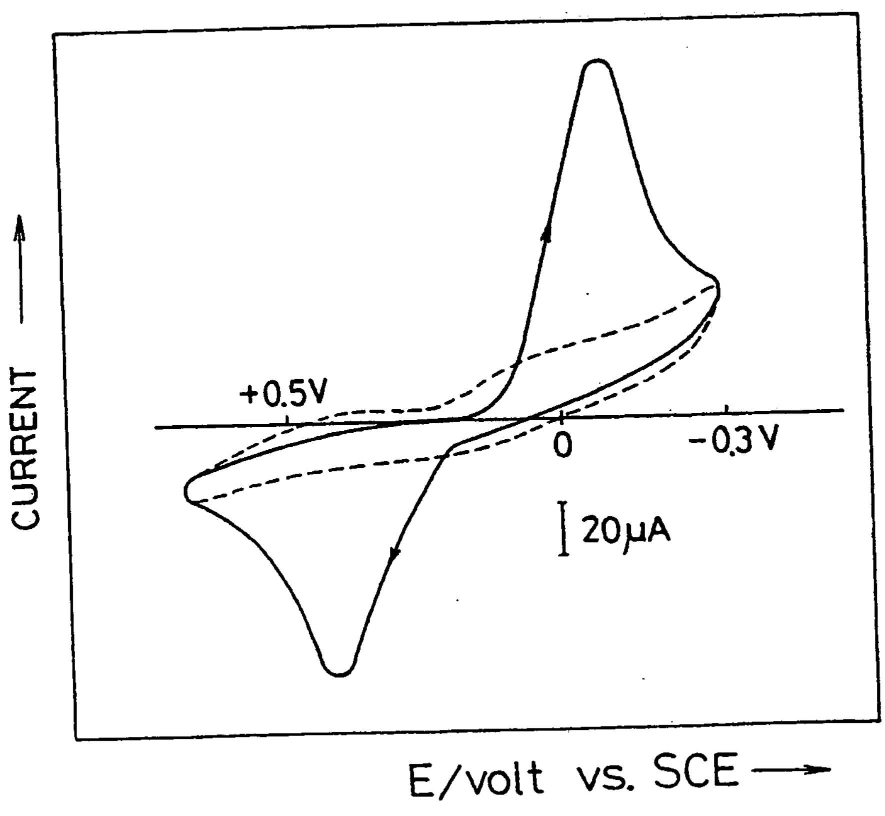

CURRENT
+0.5V
0
-0.3 V
20μA
E/volt vs. SCE

FIG. 15

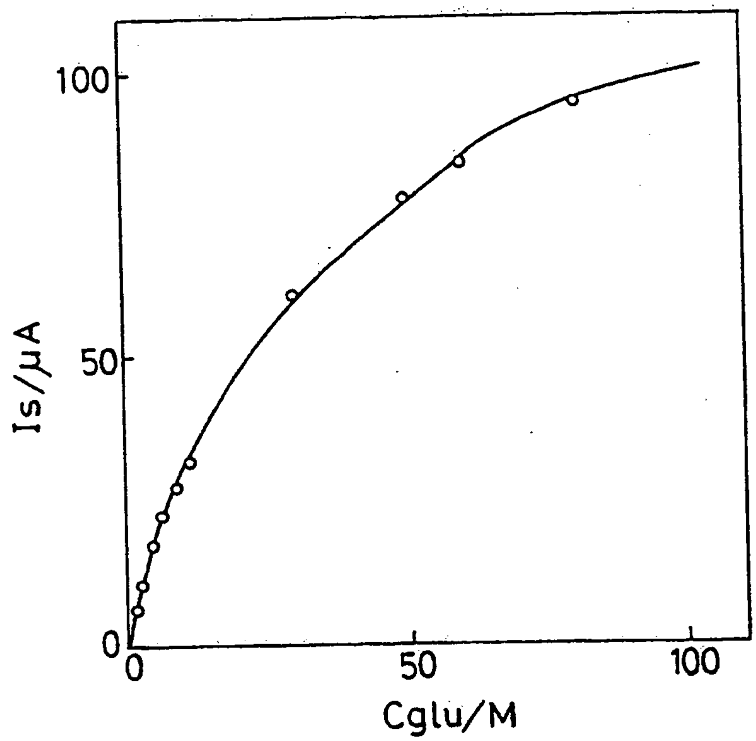


FIG. 16

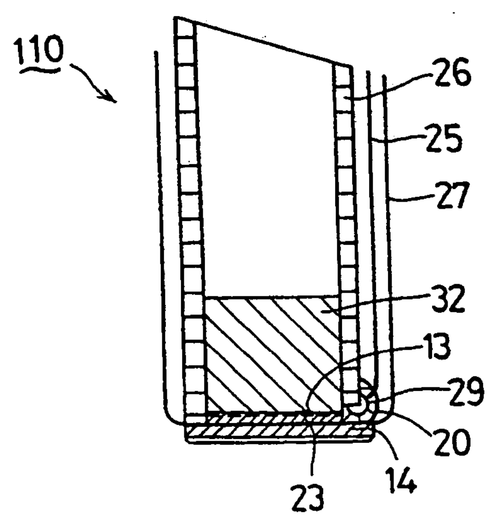


FIG. 17

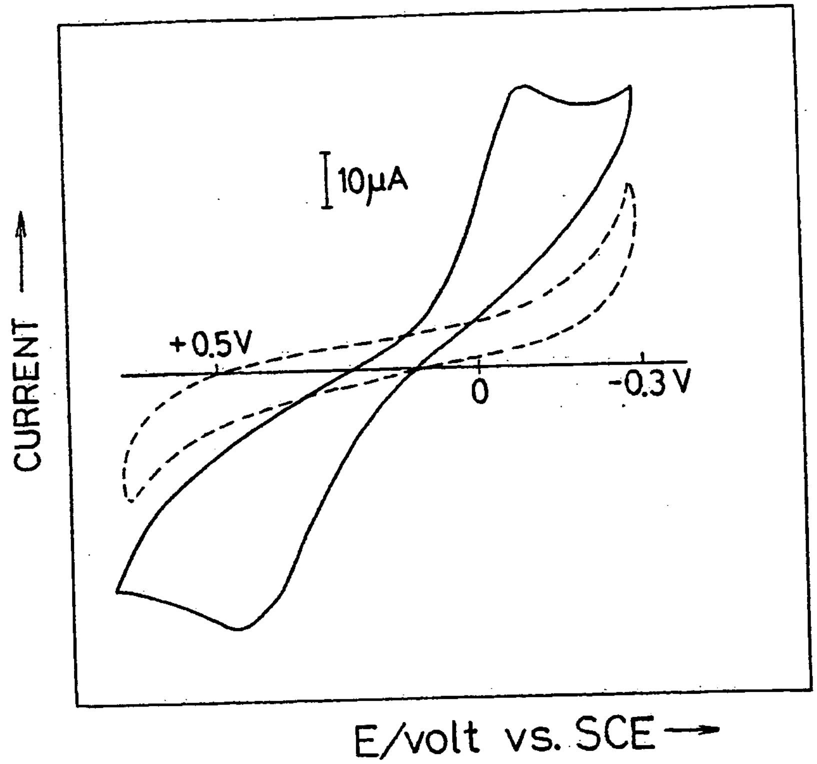

10μA
CURRENT
+0.5V
0
-0.3 V
E/volt vs. SCE

FIG. 18

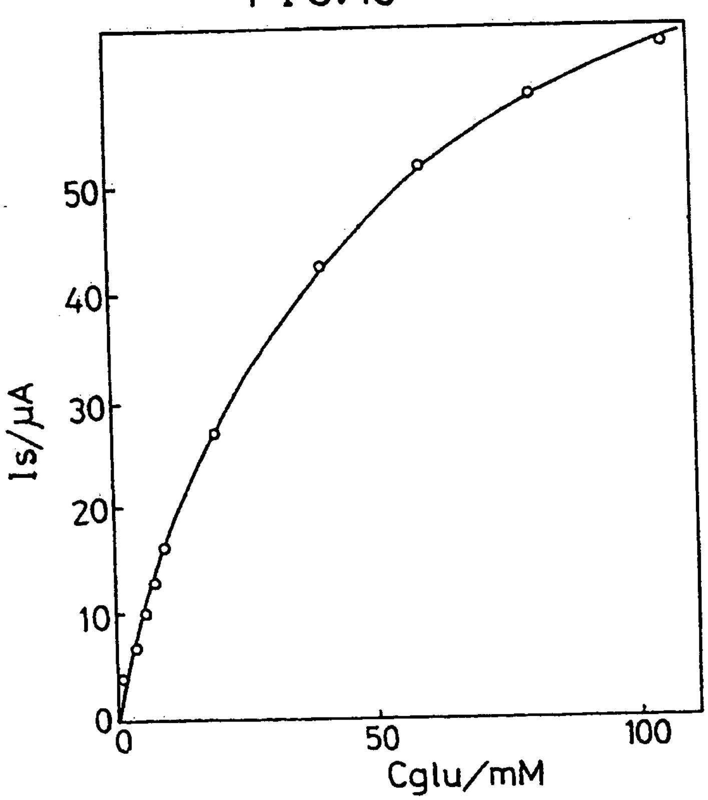

Is/μA
0
10
20
30
40
50
0
50
100
Cglu/mM

FIG. 19

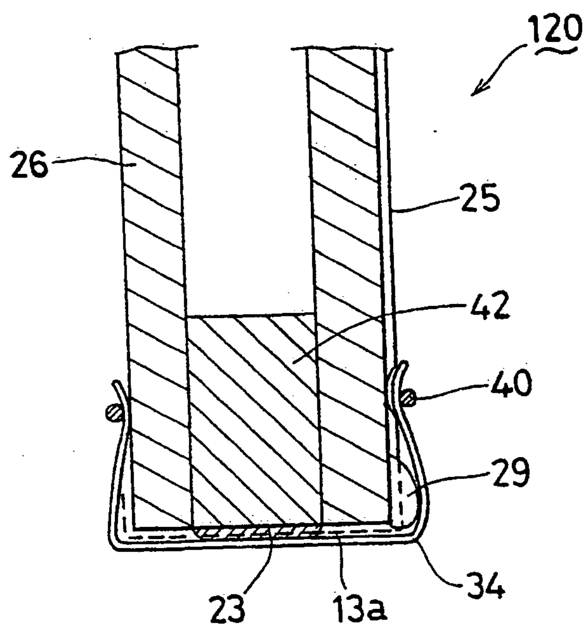

120
26
25
42
40
29
34
23
13a

FIG. 20

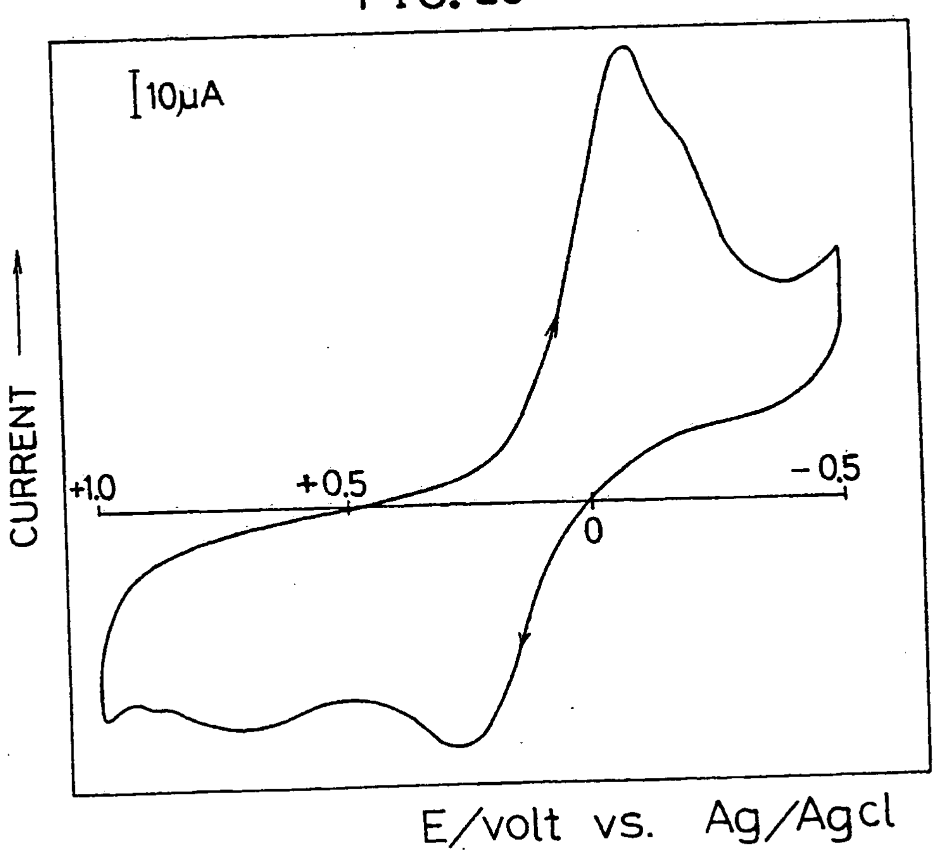

10μA
CURRENT
+1.0
+0.5
0
−0.5
E/volt vs. Ag/Agcl

FIG. 21